

Effects of Burial Depth and Substrate on the Emergence of *Bromus rubens* and *Brassica tournefortii*

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Abstract.—The germination of seed is critical in deserts where annual plants are abundant and rely on seed buried in the soil for sustaining populations. The exotic annuals *Bromus rubens* and *Brassica tournefortii* threaten arid indigenous ecosystems such as the Mojave Desert, but little is known about the potential effects on seed emergence of different burial depths and substrates that could enhance or reduce emergence. Using seed from Mojave Desert populations, we conducted a three-factor greenhouse experiment testing the effects of species (*Bromus* or *Brassica*), burial depth (0, 2, 5, or 10 cm), and substrate (none, gravel, or litter) on seed emergence. Species and substrate interacted significantly with burial depth. Both species displayed the greatest emergence when seeds were sown on the soil surface (70% emergence for *Bromus* and 52% for *Brassica*), but *Bromus* emergence declined less at a 2-cm depth than *Brassica*. Emergence of surface-sown seed did not differ significantly among substrate types, but emergence of buried seed was significantly reduced below gravel substrates compared to no substrate or litter substrates. This suggests that seed fates in the soil (such as seed mortality by germination but not emergence from the soil) can be altered by manipulating soil surface conditions.

Introduction

Seed germination is the initiation of growth by the embryo within a seed, and emergence is the appearance of a seedling above the soil surface. These processes are critical in ecosystems and influence plant regeneration, habitat structure for biota, and seed resources available to granivores (Harper 1977). Seed ecology also plays key roles in contemporary biological conservation (Baskin and Baskin 2001). One of these roles is understanding the regeneration ecology of exotic species, to potentially aid in the development of management strategies to limit the establishment of exotics in indigenous ecosystems (van der Valk and Pederson 1989).

Two of the many factors that can affect seed emergence are the type of substrate on or below which seeds are located and the depth at which seeds are buried in the soil (Scoles-Sciulla and DeFalco 2009). Seeds can be dispersed onto a variety of substrates (e.g., leaf litter versus mineral soil), which can differ in the protection they afford to seeds and their penetrability by emerging seedlings. For example, Evans and Young (1970) found that placing seeds of the annual *Bromus tectorum* (cheatgrass) below 5 or 10 cm of herbaceous leaf litter increased fall and early spring emergence by 4- to 5-fold in a field experiment in the Great Basin Desert of western Nevada. Seeds can become buried at different depths in the soil through a variety of processes, such as water movement, interment by biota, or being covered by litter or dust (Harper 1977). Chauhan et al. (2006) illustrated the effects

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of burial depth on *Brassica tournefortii* (Sahara mustard; hereafter *Brassica*) using Australian seed lots in a greenhouse experiment. Emergence was 29% for seeds sown on the soil surface, 51% for seeds buried 1 cm deep, and zero at a depth of 5 cm. Knowledge of these types of factors can facilitate an understanding about controls on the establishment patterns of plants, including exotic plants (DeFalco et al. 2009). If seeds are buried to a depth (or within a substrate) where germination but not emergence occurs, or where seeds decompose, plant establishment could be reduced. Recently, for instance, Kollmann et al. (2011) proposed burying propagules as a potential treatment for reducing *Rosa rugosa* (Japanese rose), an exotic shrub in northwestern Europe.

Exotic, invasive plant species are considered a primary threat to indigenous ecosystems in the Mojave Desert (Steers and Allen 2010). Two of the most invasive species of concern are the annual grass *Bromus rubens* (red brome; hereafter *Bromus*) and the annual forb *Brassica*. The species provide fuel that facilitates the spread of fires destructive to late-successional shrub vegetation and compete with native plants (Lei 2006). The purpose of this study was to better understand factors potentially influencing the emergence of *Bromus* and *Brassica*, in particular 0–10 cm burial depths and simulated litter, gravel, and mineral soil substrates.

Methods

The Mojave Desert is in parts of California, Nevada, Utah, and Arizona, and is classified as a hot desert that receives the majority of its precipitation in winter (Smith et al. 1997). Shrublands dominated by *Larrea tridentata* (creosote bush) and *Coleogyne ramosissima* (blackbrush) typify the physiognomy of the desert at the lower and middle elevations (Lei and Walker 1997). *Bromus* and *Brassica* can be the dominant annual plants growing below shrub canopies, but both species also can become established in open areas especially during moist years (Beatley 1966, Trader et al. 2006). Human and non-human (e.g., animal burrowing) disturbances appear to promote the establishment of these exotics, but they can invade relatively undisturbed desert (Beatley 1966, Bangle et al. 2008).

Bromus is native to southern Europe, northern Africa, and southwestern Asia, occurring from sea level to 1,300 m in these regions (Brooks 2000). The species is thought to have been introduced to North America by the mid-1800s and was well established as a dominant annual in the Mojave Desert by the mid-1950s (Brooks 2000). *Brassica* is native to the semi-arid and arid deserts of North Africa and the Middle East and to the Mediterranean lands of southern Europe. This species is believed to have been introduced to North America in the early 1900s (Minnich and Sanders 2000). Seed weights reported for *Bromus* have ranged from 1.82–2.47 mg/seed (Royal Botanical Garden Kew, Wakehurst Place, England). Trader et al. (2006) reported a mean weight of 1.17 mg/seed for *Brassica* in the Mojave-Sonoran Deserts.

We collected seed of *Bromus* and *Brassica* in the eastern Mojave Desert of southern Nevada. We harvested *Bromus* seed in April 2008 from two sites approximately 400 m in elevation in Lake Mead National Recreation Area (LMNRA) and one site (Loop Fire) 1,100 m in elevation in Red Rock Canyon National Conservation Area. *Brassica* seed was collected from three sites in LMNRA ranging in elevation from 360–390 m. The LMNRA collection sites were near 36°00'N, 114°40'W and the Loop Fire site was near 36°00'N, 115°25'W. Seeds were stored at room temperature in paper bags in a laboratory until the experiment. A weather station (Boulder City, Nevada, 768 m in elevation) centrally located within LMNRA has reported averages of 14 cm/yr of precipitation, a

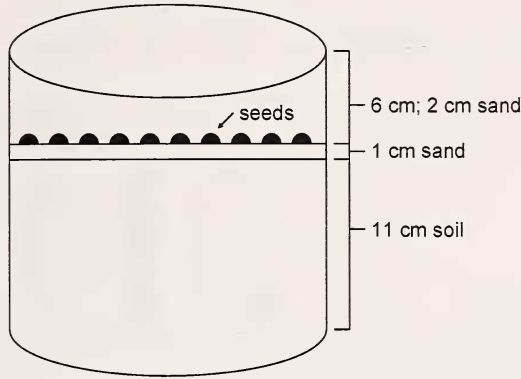


Fig. 1. Example of a treatment with a 2-cm seed burial depth, where 2 cm of sand are placed on top of the seeds. A substrate of litter or gravel would be added on top of the 2 cm of sand if required by a treatment combination.

July daily maximum temperature of 39°C, and a January daily minimum temperature of 4°C (1931–2004 records; Western Regional Climate Center 2010). The Spring Mountain Ranch State Park weather station, 7 km south of the Red Rock collection site and 1,152 m in elevation, has reported 29 cm/yr of precipitation, a July daily maximum temperature of 36°C, and a January daily minimum temperature of –1°C, averaged from 1977–2009 records (Western Regional Climate Center 2010).

The experiment was a randomized, full-factorial design consisting of three factors: two levels of species (*Bromus* and *Brassica*), four burial depths (0, 2, 5, and 10 cm), and three levels of substrate (none, litter, and gravel). Each of the 24 treatment combinations was replicated three times. We conducted the experiment in a greenhouse at the University of Nevada Las Vegas (Las Vegas, NV), with individual pots (3.8 L, 15 cm in diameter, and 18 cm tall) serving as replicates. Pots were filled with sterile potting soil (Kellogg Garden Products, Carson, CA) with 1 cm of sterile sand (all-purpose sand, supplied by Quikrete, Atlanta, GA) placed on top of the potting soil. The layer of sand was added so that seeds had contact with a sand layer resembling desert soils rather than the potting soil. Ten randomly selected seeds of the species assigned to a pot were evenly sown on top of the sand layer. Burial treatments entailed covering the seeds with additional sand so that seeds were buried to the appropriate experimental depths. The 0-cm level of the burial treatment had no sand added on top of the seeds and represented seeds simply sown on the surface. A substrate, if part of the treatment combination, was then placed on top of the seed (for the 0-cm burial treatment) or on top of the seed covered by the sand in the 2-, 5-, and 10-cm burial treatments (Fig. 1). For the substrate litter treatment, dead thatch material of approximately equal *Bromus* and *Brassica* was obtained from the seed-collection sites and shaken to remove unwanted material. Approximately 5 g of thatch were placed in a layer ca. 1 cm thick. We used a 1-cm thick layer of gravel (Quikrete all-purpose gravel, consisting of pebbles ca. 1 cm in diameter) for the gravel substrate.

We designed the treatments to mimic substrates to which seeds could be exposed in the field. Seeds in soil below shrubs must penetrate through litter to emerge, whereas seeds in openings must emerge on mineral soil, situations simulated by the litter and no substrate treatments. The gravel substrate treatment was selected to mimic coverage of seeds by mechanisms such as biopedturbation by small mammals (Whitford and Kay 1999) or

Table 1. Analysis of variance results for the influences of burial depth (0, 2, 5, or 10 cm) and substrate (none, litter, or gravel) on the emergence of seeds of *Bromus rubens* and *Brassica tournefortii* collected in the Mojave Desert.

Effect	Num DF ^a	Den DF ^a	F-statistic	P
Species	1	48	3.47	0.0687
Depth	3	48	44.69	<0.0001
Substrate	2	48	8.45	0.0007
Species × depth	3	48	3.69	0.0181
Species × substrate	2	48	0.39	0.6794
Depth × substrate	6	48	2.66	0.0262
Species × depth × substrate	6	48	0.75	0.6108

^a Numerator and denominator degrees of freedom.

deposition in washes. Both species can inhabit washes in the Mojave Desert (Beatley 1966).

The experiment began in March 2009, when germination and emergence of the species occur in the field (Beatley 1966). We randomly arranged pots on a bench in a greenhouse maintained at a constant 24°C without supplemental lighting. Pots were hand watered to soil moisture capacity every two or three days and emergents were counted weekly for two months.

We analyzed the data as a fully crossed, three-factor experiment with the proportion of seeds emerging (out of 10 for each pot) as the response variable in an analysis of variance. We used PROC MIXED with Tukey adjustments for multiple comparisons in SAS software (SAS Institute 1999). Because some of the treatment combinations had no emergence and hence zero variance, we compared results of this model with *P* values computed through permutation using Cassell's (2002) macro for SAS. Permuted and normal approximation *P* values were identical to the hundredths place, so we report the *P* values from the normal approximation model.

Results

Species and substrate both interacted significantly with burial depth in influencing seed emergence (Table 1). Emergence of both species at a 0-cm (surface) depth was significantly greater than at 5- or 10-cm depths (Fig. 2). The species × depth interaction was related to the finding that *Bromus*' emergence at a 2-cm depth did not differ from its surface emergence, whereas *Brassica* had significantly lower emergence at 2 cm than at the surface. Similarly, emergence of surface-sown seeds did not differ among substrates, but seeds buried at a 2-cm depth exhibited sharp declines in emergence when gravel substrates were present. Only the litter treatment had appreciable seed emergence at 5- or 10-cm depths, albeit at levels significantly reduced compared to surface-sown seeds.

Discussion

Burial at depths > 2 cm and below gravel substrates sharply curtailed emergence of both *Bromus* and *Brassica*. However, *Bromus* displayed a stronger ability to emerge at a 2-cm depth than *Brassica*, resulting in a significant species × depth interaction (Fig. 2). Seeds of *Bromus* are 1.5- to 2-fold heavier than those of *Brassica* (Royal Botanical Garden Kew, Wakehurst Place, England; Trader et al. 2006). These seed weight-emergence patterns are consistent with the common observation that heavier seeds typically can emerge from greater depths than lighter seeds (Harper 1977).

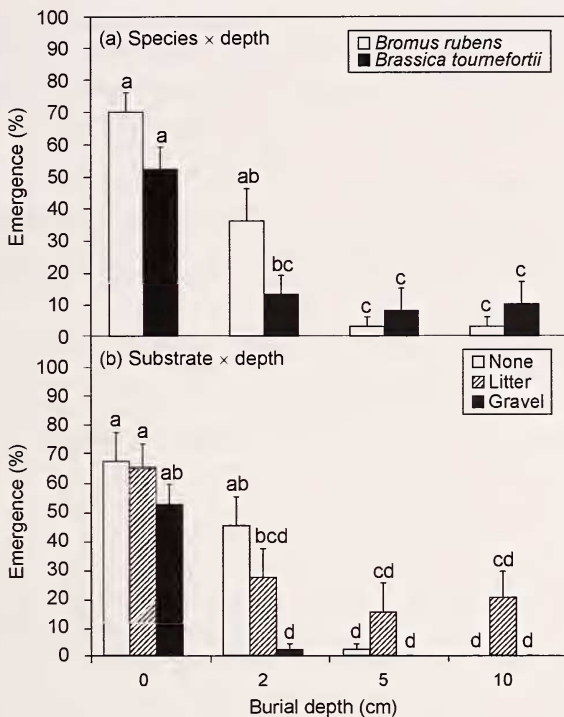


Fig. 2. Mean emergence of two annual plant species of the Mojave Desert in relation to burial depth and substrate treatments, for significant interactions shown in Table 1. Error bars are standard errors of the means. Means without shared letters, within (a) or (b), differ at $P < 0.05$.

While the literature is limited on burial and substrate effects on the emergence of these species, some previous studies of these and related species do permit comparisons with this experiment. Our results agree with a previous greenhouse experiment with Australian populations of *Brassica* where emergence was negligible when seeds were buried at depths of ≥ 3 cm (Chauhan et al. 2006). However, our results are incongruent with Chauhan et al.'s (2006) finding that more *Brassica* seeds emerged from 1–2 cm burial depths than on the surface, or the findings of Thanos et al. (1991) that more seeds of Greek populations of *Brassica* emerged when buried than at the soil surface. We found that emergence was greater for seeds sown on the surface than when buried at any depth. Thanos' et al. (1991) study was conducted outdoors, however, where exposure of seeds to weather and granivory (especially on the soil surface) could have reduced the seeds available to germinate, although it was not mentioned if seeds were open to predation by animals or invertebrates. These types of factors could have large effects on plant establishment in the field, where burial of the seeds below some soil could reduce granivory and protect seeds (DeFalco et al. 2009).

We found that litter had a minimal influence on *Bromus* or *Brassica* emergence, consistent with the observation that these species can become established below perennial plants in the Mojave Desert where litter accumulates (Strojan et al. 1979). What remains uncertain, however, is whether effects of different types of litter from different perennial species vary, or if depths of litter in the field could become sufficiently thick to hinder emergence. Working with *Bromus tectorum* in *Pinus ponderosa* (ponderosa pine) forests using a greenhouse experiment, Pierson and Mack (1990), similarly to our study, found

that emergence with and without 1.5 cm of *P. ponderosa* litter did not differ. However, a thicker litter layer, 6 cm, decreased emergence by 36%. In contrast, Evans and Young (1970) placed *B. tectorum* seeds below 5 or 10 cm of herbaceous litter in a field experiment in western Nevada's Great Basin Desert and found that emergence increased compared to without litter by 4- to 5-fold in fall and early spring. Litter could switch from positive (such as protecting seeds; DeFalco et al. 2009) to negative effects on seed emergence based on a variety of factors, such as the thickness or chemical composition of the litter. Further manipulating litter through field experiments that include different thicknesses of litter could be useful for assessing the potential role of litter in the establishment of exotic plants in the Mojave Desert.

Most of the burial depth research with annual *Bromus* species has concentrated on different questions than our focus on emergence. Several studies with *Bromus tectorum*, for example, have examined effects of burying packets or capsules filled with seeds on the viability and germinability of the seed when exhumed (e.g., Gleichsner and Appleby 1989, Burnside et al. 1996, Wicks 1997). These are central questions about the longevity of seed banks, but do not address depth effects on emergence *per se*. Findings from these studies suggest that *B. tectorum* seeds can germinate *in situ* (though not necessarily emerge) when deeply buried. Gleichsner and Appleby (1989), for instance, reported that 87% of seeds germinated within 30 days when buried at a depth of 30 cm in an agricultural field in western Oregon. These findings suggest that seeds in our experiment possibly germinated at the deep burial depths, but were not able to emerge, although this is not known with certainty for our study species.

The observed emergence patterns in the greenhouse of these species may reinforce some field patterns and highlight ideas that could benefit from further research. For instance, the finding that litter placed on top of seeds at the 0- and 2-cm burial depths did not influence emergence, relative to the no substrate treatment, supports the observation that seedlings of these species are abundant below perennial shrubs that have litter layers in the Mojave Desert (Strojan et al. 1979, Brooks 2009, Abella et al. 2011). It should be noted, however, that intrinsic effects of litter could interact with shade or other effects of the shrubs, vary with the type of litter, and differ in open- versus below-shrub environments. Nevertheless, both of our study species demonstrated the ability to emerge through a layer of their own litter at the 1-cm litter thickness we tested. In contrast, gravel as a substrate sharply decreased emergence at any burial depths ≥ 2 cm. This effect could partly relate to the added depth of dense material from the gravel that seeds needed to penetrate up through, as opposed to the low-density litter layer. Working with the native Mojave Desert perennial *Pleuraphis rigida* (big galleta grass) in a greenhouse experiment, Winkel et al. (1995) concluded that burial below 2–3 cm of gravel aided emergence, whereas thicker layers of 4–5 cm curtailed emergence. *Bromus* and *Brassica* occupy many different soil types in the Mojave Desert, but specifically testing soil particle size effects on emergence when seeds are sown on top of or below materials differing in particle size could help further isolate potential substrate influences. Results indicate that approximately 5 cm is the greatest burial depth from which reliable emergence can be expected for either exotic species.

Effects of the burial depths and gravel substrates support suggestions that a possible control treatment for the species is to bury seeds (or cover them with a substrate) to a depth where germination, but not emergence, occurs, or where viability is lost due to seed decomposition (Wicks 1997). This could deplete the seed bank over time. Gleichsner and Appleby (1989), for instance, suggested that tillage be used to bury seeds in western

Oregon agricultural fields for reducing *Bromus tectorum* emergence. Tillage is not necessarily practical in intact desert ecosystems due to non-target impacts on native vegetation and soils. However, the data do suggest a possibility that mulches (e.g., wood chips) or similar materials may form physical barriers to emergence.

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